METHODS, SYSTEMS, AND USES FOR CALIXARENES

BACKGROUND OF THE INVENTION

[0001]   The present invention relates to the general field of chemical sensing, and more particularly to calixarene compositions, devices and methods of use for identifying and detecting the presence of nitrogen oxide compounds in gas/vapor or liquid mixture.

5   [0002]   Nitrogen oxide compounds, the so-called NO_X gases—a collective term that refers to the family of oxides of nitrogen—are toxic atmospheric pollutants derived generally from fossil fuel combustion, power plants, and large-scale industrial processes. NO_X are involved in the formation of ground-level ozone, participate in global warming, and also form toxic chemicals, nitrate particles and acid rain/aerosols. In addition, NO_X are active components in various harmful nitrosation processes in biological tissues. Free radical NO rapidly reacts with oxygen, producing N_2O_3 (e.g., $NO \cdot NO_2$) and NO_2/N_2O_4 that are powerful nitrosating agents, both in the gas phase and in solution.

[0003]   The pathophysiologic significance of NO_X derives from their ability to generate mutagenic nitrosopeptides and diazopeptides, to produce carcinogenic nitrosoamines, and to nitrosate and deaminate DNA nucleobases. According to The United States Environmental Protection Agency, national emissions of NO_X have increased over the past 20 years by 4% and continue to rise. With the increase in NO_2 circulation in the atmosphere, there remains a need to not only systematically monitor nitrogen levels in the atmosphere, and to develop improved methods of fixation and utilization of nitrogen oxide compounds.

[0004]   The present invention addresses many of the needs mentioned above as well as other objectives that will be appreciated by those skilled in the art.

SUMMARY OF THE INVENTION

[0005]   The present invention solves many problems associated with current increases in

5   NO_x levels in the atmosphere. The present invention provides new and improved methods of detecting and identifying of NO_X compounds, provides new and improved uses of these compounds as well as methods for storage of NO_X compounds. New and improved compounds for use in such applications are also provided.

[0006]   In accordance with one aspect of the present invention, a method of and device for

10   identifying, detecting and/or chemically utilizing NO_X compounds is provided, wherein simple calixarenes, such as calix[4]arenes (e.g., tetra-O-alkylated calix[4]arenas) are exposed to nitorogen oxides capable of producing nitrosonium ions (i.e. NO^+) and form calixarene-nitrosonium complexes that are readily identified. Importantly, the process of forming one or more calixarene-nitrosonium complexes is reversible. In addition, the calixarene-

15   nitrosonium decolor with the addition of anything that reacts with the nitrosonium ion (e.g., water, alcohol) enabling the parent calixarenes (e.g., calix[4]arenes) to be recovered.

[0007]   In accordance with another aspect of the present invention, a method of and device for attaching functionalized calix[4]arenes to a solid support (e.g., silica gel) is provided resulting in a solid material capable of identification, detection and/or entrapment of NO_X

20   compounds. As such nitrogen oxide compounds may be readily purified by means of the present invention.

[0008]   Further, in accordance with the present invention, a method of and device for using calixarene-nitrosonium complexes is provided for the transfer of NO^+ for nitrosation reactions. As such, novel nitrogen oxides storing materials are also provided.

25   [0009]   An advantage of the present invention is that it provides a new and improved charge-transfer interaction for NO_X compounds (NO_x) allowing the compounds to be readily

detected in the presence of other gases, including H_2O, O_2, HCl, SO_X, NH_3, and even NO. In addition, the NO_X compounds and complexes formed with the present invention are vehicles for nitrosonium transfer and for encapsulated nitrosating reagents. The complexes also serve as nitrogen oxide (e.g., NO_2/NO_X) detection, sensing materials.

[00010]   Those skilled in the art will further appreciate the above-mentioned advantages and superior features of the invention, together with other important aspects thereof upon reading the detailed description that follows in conjunction with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[00011]   For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying FIGURES in which corresponding numerals in the different FIGURES refer to corresponding parts and in which:

FIGURE 1 depicts an example of chemical fixation of NO_2/N_2O_4 with calix[4]arene and formation of an encapsulated nitrosonium complex;

FIGURE 2 depicts two methods of preparing nitrosonium complexes of calix[4]arene compound 1 with (Left) addition of $NO^+SbF_6^-$ to compound 1 in chloroform, (Right) the reaction of NO_2/N_2O_4 with compound 1 in the presence of $SnCl_4$ in chloroform and (Below) MacroModel representation of an NO^+ encapsulation process, wherein the CH hydrogen and long alkyl chains are omitted from view;

FIGURE 3 depicts two methods of synthesizing nitrosonium complexes with a MacroModel representation of an NO^+ encapsulation process, wherein the CH hydrogen and long alkyl chains are omitted from view;

FIGURE 4 depicts an example of mesitylene-derived calixarene compound 6 that does not encapsulate NO^+ showing a space-filling representation of compound 6 without the long alkyl chains;

FIGURE 5 depicts portions of the ^{1}H NMR spectra (500 MHz, CDCl$_3$, 295 ± 1 K) of (A) calix[4]arene compound **1**, (B) nitrosonium complex **7**, wherein the identical spectrum was obtained upon addition of NO$^+$SbF$_6^-$ to compound **1**, (C) calix[4]arene compound **2** and (D) nitrosonium complex **8**, wherein the identical spectrum was obtained upon addition of

5   NO$^+$SbF$_6^-$ to compound **2** and, wherein the residual CHCl$_3$ signals are marked with a dark circle;

FIGURE 6 depicts a host-guest dynamic in a calix[4]arene-NO$^+$ complex;

FIGURE 7 depicts NO$^+$ transfer between calixarene containers and formation of complex **9** from complex **8**;

10   FIGURE 8 depicts an example of a nitrosonium transfer experiment with portions of the UV-vis spectra;

FIGURE 9 depicts portions of the ^{1}H NMR spectra (500 MHz, CDCl$_3$, 295 ± 1 K) of (A) nitrosonium complex **8**, obtained from compound **2**, NO$_2$ and SnCl$_4$, (B) mixture of complex **8** and calixarene compound **5** after 20 minutes with about 30% conversion to

15   complex **9**, (C) the same mixture after 1 hour, wherein complex **9** is formed with >95% conversion, and (D) nitrosonium complex **5**•NO$^+$SbF$_6^-$, independently obtained from compound **5** and NO$^+$SbF$_6^-$;

FIGURE 10 depicts a preparation of calix[4]arene functionalized silica gel **15** with (A) Et$_3$N, THF, rt, 12 h and an example of NO$_2$ entrapment chromatography experiment; and

20   FIGURE 11 depicts an *N*-Nitrosation reaction using secondary amides **16a-c** with complex **8** to form *N*-nitrosoamides **17a-c** and shows two prereactive complexes as examples.

DETAILED DESCRIPTION OF THE INVENTION

[00012]   Although making and using various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many inventive concepts that may be embodied in a wide variety of contexts. The specific aspects

5   and embodiments discussed herein are merely illustrative of ways to make and use the invention, and do not limit the scope of the invention.

[00013]   Nitrogen oxide compounds, also referred to as NO_X gases are toxic atmospheric compounds as well as pathophysiologic compounds. As used herein, NO_x refers to any mixture of oxides of nitrogen capable of generating a nitrosonium ion. One of the most

10   prevalent of these compounds is NO_2, a paramagnetic gas since it has an unpaired electron on the nitrogen. It has an intense brown-orange color. When reacting with itself, it forms a colorless dimer, dinitrogen tetroxide (N_2O_4). For N_2O_4, there is a very weak N-N bond and, at higher temperatures, the gas rapidly dissociates back to NO_2. The position of equilibrium between the two compounds (N_2O_4 and NO_2) and the color of the system varies with

15   temperature. Below –21 degrees Centigrade, only pure, solid N_2O_4 is present. Above 140 degrees Centigrade the system is 100% NO_2. This dynamic interconversion between NO_2 and N_2O_4 makes it impossible to study either of species alone. Of particular importance for mammals is that N_2O_4 may disproportionate to ionic $NO^+NO_3^-$.

[00014]   Calix[4]arenes are building block-like molecules and have been used in the

20   construction of molecular containers (e.g., cavitands, (hemi)carcerands, and capsules). *Cone*-shaped calix[4]arenes are about 4 Å deep and about 7 Å in diameter at the upper rim. Tetra-*O*-alkylated *cone* calix[4]arenes exist in the pinched C_{2v} symmetrical conformation, with two opposite aromatic rings almost parallel and situated about 5 Å apart, and two others flattened. This conformation is more preferable than the perfect cone C_{4v} conformation. Usually, the

25   interconversion between two C_{2v} structures is fast on the NMR time scale. Calix[4]arenes in a *1,3-alternate* conformation are much more rigid and possess a cylindrical inner tunnel, defined by two cofacial pairs of aromatic rings oriented orthogonal along the cavity axis. According to a number of X-ray studies, this tunnel is about 5-6 Å in diameter.

[00015] Complexes of calix[4]arenes with neutral molecules are weak. The cavities are obviously too small and they lack additional binding sites. In most of the crystal structures of the inclusion complexes of calixarenes, the guest molecule (i.e., the molecule that forms a complex with a calixarene) is positioned not inside, but roughly above the plane defined by the upper carbon atoms of the cyclic polyaromatic skeleton. Cations are known to interact with more strength with a calixarene π-surface. Ammonium ions and metal cations have been found to complex within the cone-shaped cavities. *1,3-Alternates*, functionalized with appropriate binding sites on the phenol oxygens, bind metal cations (e.g., Na^+, K^+, and Ag^+) both with "hard" oxygens and "soft" π-basic aromatic rings.

[00016] The present invention has found that interactions between calix[4]arenes and nitrogen oxide compounds leads to strong calixarene-nitrosonium complexes. FIGURE 1 shows the host-guest complexes formed upon interaction between NO_x and simple calix[4]arenes. In fact, calixarene complexes of the present invention are identified by: (1) an ability to react with NO_x to form stable nitrosonium (NO^+) complexes; (2) identification and detection (e.g., colorization) of the calixarene-NO^+ complexes; and (3) dissociation of the identified calixarene-NO^+ complexes (e.g., decoloration of the complex via anything that reacts with the nitrosonium ion, such as water or alcohol, as examples). Importantly, the calixarene-NO^+ complexes may be used for the transfer of NO^+ compounds, nitrosation reactions, NO_x identification, NO_x detection, and NO_x sensing, as well as serving as supramolecular NO^+ storing materials.

General Procedures

[00017] General methods of the present invention are described here and include those in Zyryanov G., et al. JACS. 2003; 125:2997-3007 (incorporated herein by reference). Melting Points were determined on a Mel-Temp apparatus (Laboratory Devices, Inc.) and a Buchi apparatus. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 295 ± degrees Centigrade, unless stated otherwise, on JEOL Eclipse 500 MHz spectrometer. Chemical shifts were measured relative to residual non-deuterated solvent resonances. FTIR spectra were recorded on a Bruker Vector 22 FTIR spectrometer. UV-vis spectra were measured on a JASCO V-

530 spectrophotometer. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF) was performed on a Bruker BiFLEX I linear time-of-flight mass spectrometer operated in delayed extraction mode. Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer. For column chromatography, Silica Gel 60 Å (Sorbent

5   Technologies, Inc.; 200–425 mesh) was used. All experiments with moisture- or air-sensitive compounds were run in freshly distilled, anhydrous solvents under a dried nitrogen atmosphere. Molecular modeling was performed generally using MacroModel 7.1.

[00018]   Parent tetrahydroxycalix[4]arenes were prepared according to procedures known to one of ordinary skill in the art (see e.g., Gutsche, C. D., Iqbal, M. *Org. Synth.* 1990, Vol. 68,

10   pp. 234-237). NO_2/N_2O_4 was generated from copper and concentrated HNO_3 or sodium nitrate and concentrated H_2SO_4.

Example for Preparing 25,26,27,28-Tetra (*n*-hexyloxy)calix[4]arene-1,3-alternate (compound 2).

[00019]   To a suspension of 25,27,26,28-tetrahydroxycalix[4]arene (about 4.24 g, 0.01 mol)

15   and K_2CO_3 (about 4.2 g, 0.03 mol) in MeCN (about 200 mL) *n*-hexylbromide (about 4.2 mL, 0.03 mol) was added, and the reaction mixture was refluxed under nitrogen for about 48 hours. The precipitate was filtered off, and the solution evaporated to dryness. The residue was redissolved in CH_2Cl_2 (about 200 mL), and the solution was washed with water (3 x 150 mL) and dried over $MgSO_4$. After evaporation, the solid residue was treated with MeOH

20   (about 200 mL) to yield pure 25,27-bis(*n*-hehyloxy)-26,28-hydroxycalix[4]arene as a white solid (5.0 g, 84%). ^1H NMR results: δ 8.23 (bs, 2 H), 7.06 (d, *J* = 8 Hz, 4 H), 6.91 (d, *J* = 8 Hz, 4 H), 6.73 (t, *J* = 8 Hz, 2 H), 6.65 (t, *J* = 8 Hz, 2 H), 4.32 (d, *J* = 13 Hz, 4 H), 4.00 (t, *J* = 7 Hz, 4 H), 3.37 (d, *J* = 13 Hz, 4 H), 2.1-2.0 (m, 4 H), 1.75-1.7 (m, 4 H), 1.45-1.4 (m, 8 H), 0.95 (t, *J* = 7 Hz, 6 H).

25   [00020]   To a suspension of 25,27-bis(*n*-hehyloxy)-26,28-hydroxycalix[4]arene (about 5.92 g, 0.01 mol) and Cs_2CO_3 (about 50 g, 0.15 mol) in MeCN (about 300 mL) *n*-hexylbromide (about 5.74 mL, 0.04 mol) was added, and the reaction mixture was refluxed under nitrogen for about 48 hours. After cooling, the precipitate was filtered off and treated with a mixture

of water (about 100 mL) and CH$_2$Cl$_2$ (about 100 mL). The organic layer was separated, washed with water (2 x 100 mL), dried over MgSO$_4$, and evaporated. The residue was recrystallized from MeOH-CHCl$_3$, 10:1 to give pure compound **2** (3.50 g, 46%). Melting Point: 119 degrees Centigrade. ^{1}H NMR results were: δ 6.92 (d, J = 7.5 Hz, 8 H), 6.68 (t, J = 7.5 Hz, 4 H), 3.62 (s, 8 H), 3.54 (t, J = 7.5 Hz, 8 H), 1.89 (m, 8 H), 1.35 (m, 24 H), 0.85 (t, J = 7 Hz, 12 H). MALDI-TOF MS, m/z 783.9 ([M+Na$^+$], calculated for C$_{52}$H$_{72}$O$_4$ 783.9). Anal. calculated for C$_{52}$H$_{72}$O$_4$: C, 82.06; H, 9.53. Found: C, 81.61; H, 9.58.

Example for Preparing 5,11,17,23-Tetrabromo-25,26,27,28-tetrakis(*n*-hexyloxy)calix[4]arene-1,3-alternate (compound **3**).

[00021]   *N*-Bromosuccinimide (about 3.0 g, 0.017 mol) was added to a suspension of calixarene **2** (about 2.0 g, 2.6 mmol) in acetone (about 500 mL), and the mixture was stirred at room temperature for about 48 hours and exposed to the laboratory light. The formed solid was filtered off, washed with acetone (about 2 x 100 mL) and used in the next step without further purification. Yield 1.5 g (53 %). ^{1}H NMR results were: δ 7.12 (s, 8 H), 3.56 (s, 8 H), 3.52 (t, J = 7.5 Hz, 8 H), 1.6-1.4 (m, 8 H), 1.4-1.3 (m, 24 H), 0.93 (t, J = 7 Hz, 12 H).

Example for Preparing 5,11,17,23-Tethydroxy-25,26,27,28-tetrakis(*n*-hexyloxy) calix[4]arene-1,3-alternate (compound **4**).

[00022]   To a solution of tetrabromo derivative compound **3** (about 0.9 g, 0.83 mmol) in freshly distilled over Na and oxygen-free THF (about 150 mL) *n*-BuLi (about 30 mL of 2M solution in pentane, 60 mmol) was added at –78 degrees Centigrade, and the mixture was stirred at this temperature for about 75 minutes. Trimethyl borate (about 14 mL, 145 mmol) was then added at –78 degrees Centigrade, and the mixture was allowed to warm to room temperature. After about 5 hours, the reaction mixture was cooled down again to –78 degrees Centigrade, and H$_2$O$_2$ (about 15 mL of 30% aqueous solution) and NaOH (about 35 mL of 3N aqueous solution) was added. The resulting solution was stirred overnight at room temperature, after which the precipitate was filtered off. The mother liquor was cooled to 0 degrees Centigrade and treated with NaS$_2$O$_3$ (about 25 g), after which the mixture was filtered and the filtrate was concentrated in vacuo. The residue was treated with about 5% aqueous HCl (100 mL), and the colorless precipitate of compound **4** was filtered off and

washed with MeOH (about 50 mL). Yield 0.274 g (40%). Melting Point: 230 degrees

Centigrade. ^{1}H NMR results were (DMSO-d_6): δ 8.39 (bs, 4 H), 6.34 (s, 8 H), 3.50 (s, 8 H),

3.02 (t, J = 7.5 Hz, 8 H), 1.3-1.25 (m, 8 H), 1.25-1.1 (m, 16 H), 1.1-1.0 (m, 8 H), 0.87 (t, J =

7 Hz, 12 H). MALDI-TOF MS, m/z 847 ([M+Na$^+$], calculated for $C_{52}H_{72}O_8$ 847).

5   Example for Preparing 5,11,17,23,25,26,27,28-Octa(n-hexyloxy)calix[4]arene-1,3-alternate
(compound 5).

[00023]   To a suspension of compound 4 (about 0.3 g, 0.36 mmol) and NaH [about 0.15 g

of about 60% (wt.) suspension in mineral oil, 3.6 mmol] in freshly distilled DMF (about 50

mL) n-hexylbromide (about 0.46 mL, 3.2 mmol) was added, and the reaction mixture was

10   stirred at about 70 degrees Centigrade under nitrogen for about 24 hours. The precipitate was

filtered off, and the mother liquor was treated with a mixture of crushed ice (about 50 g),

water (about 50 mL) and CH_2Cl_2 (about 100 mL). The organic layer was separated, washed

with water (2 x 100 mL), dried over $MgSO_4$ and evaporated. The residue was recrystallized

from MeOH-CHCl$_3$, 10:1 to yield calixarene compound 5 (0.35 g, 85 %). Melting Point: 11

15   degrees Centigrade. ^{1}H NMR results were: δ 6.55 (s, 8 H), 3.83 (t, J = 7 Hz, 8 H), 3.62 (s, 8

H), 3.35 (t, J = 7 Hz, 8 H), 1.7 (m, 16 H), 1.4-1.2 (m, 48 H), 0.89 (t, J = 7 Hz, 24 H). ^{13}C

NMR results were: δ 153.6, 150.7, 134.4, 115.2, 71.6, 68.1, 37.8, 32.3, 31.8, 29.8, 26.0, 25.9,

22.9, 22.7, 14.3, 14.1; MALDI-TOF MS, m/z 1182 ([M+Na$^+$], calculated for $C_{76}H_{120}O_8$

1183).

20   Example for Preparing Tetrakis(O-n-hexyloxy)cyclophane (compound 6).

[00024]   NaH (about 0.11 g of about 60% suspension in mineral oil, 2.7 mmol) was added

to the solution of Pappalardo's cyclophane (about 0.2 g, 0.34 mmol prepared using methods

known in the art) in freshly distilled DMF (about 20 mL), and the mixture was stirred under

nitrogen for about 30 minutes. n-Hexylbromide (about 0.3 mL, 2.04 mmol) was then added,

25   and the reaction mixture was stirred at about 70 degrees Centigrade for 3 days. The

precipitate was collected and dissolved in CH_2Cl_2 (about 20 mL). The solution was washed

with water (3 x 20 mL), dried over $MgSO_4$ and evaporated. The residue was recrystallised

from MeOH-CHCl$_3$ to afford compound 6 as a white powder. Yield 0.157 g (50 %). ^{1}H

NMR results were: δ 3.89 (s, 8 H), 3.58 (t, J = 7 Hz, 8 H), 2.30 (s, 24 H), 1.8-1.7 (m, 8 H),

1.6-1.5 (m, 16 H), 1.4-1.35 (m, 8 H), 1.07 (s, 12 H), 0.91 (t, J = 7 Hz, 12 H). ^{13}C NMR

results were: δ 153.9, 138.4, 131.4, 126.7, 73.2, 32.8, 32.0, 30.4, 26.0, 22.8, 17.7, 14.2, 13.7.

Anal. calculated for $C_{64}H_{96}O_4$: C 82.70; H 10.41. Found: C, 82.37; H, 10.25.

5   Example for Preparing Calix[4]arene-nitrosonium Complexes.

[00025]   In one example, stock solutions of NO_2 were freshly prepared upon bubbling

through $CHCl_3$; the gas concentration was determined gravimetrically. In a typical

procedure, a solution of calixarene compound **1** (about 1 equivalent) in dry, freshly distilled

$CHCl_3$ was mixed with the stock solution of NO_2 (about 3 equivalent) in $CHCl_3$ and $SnCl_4$

10   (about 1.5 eq) at room temperature. After 1 hour, complex **7** was precipitated upon addition

of hexane, filtered off, washed with hexane (about 2 times), and dried in vacuo. ^{1}H NMR

results were: δ 6.99 (s, 8 H), 4.39 (d, J = 13 Hz, 4 H), 4.02 (t, J = 7.5 Hz, 8 H), 3.44 (d, J =

13 Hz, 4 H), 2.0-1.9 (m, 8 H), 1.5-1.3 (m, 24 H), 0.93 (t, J = 7 Hz, 12 H); UV-vis ($CHCl_3$):

λ_{max} 563; FTIR ($CDCl_3$): ν 1923 (NO^+) 1461, 1298, 1047 (NO_3^-); Anal. calculated for

15     $C_{68}H_{104}O_4 \cdot NO^+NO_3^- \cdot 1.8SnCl_4$: C, 52.81; N, 1.81; H, 6.78. Found: C, 52.60; N, 1.67; H, 7.55.

Complex **8** was obtained analogously and ^{1}H NMR results were: δ 7.17 (t, J = 7.5 Hz, 4 H),

7.08 (d, J = 7.5 Hz, 8 H), 3.87 (t, J = 7.5 Hz, 8 H), 3.60 (s, 8 H), 1.9-1.8 (m, 8 H), 1.4-1.3 (m,

24 H), 0.93 (t, J = 7 Hz, 12 H); UV-vis ($CHCl_3$): λ_{max} 524 nm; FTIR ($CDCl_3$): ν 1955 (NO^+),

1438, 1246 1901, (NO_3^-); Anal. calculated for $C_{52}H_{72}O_4 \cdot NO^+NO_3^- \cdot 1.5SnCl_4$: C, 50.21; N,

20   2.25; H, 5.83. Found: C, 50.23; N, 1.82; H, 5.99. Complex **9** is generally not prepared using

this protocol; because, only dealkylation/oxidation products were detected. For spectroscopic

characterization, complex **9** was generated in the exchange experiment between nitrosonium

complex **8** and free calixarene compound **5**, as depicted in FIGURE 7.

[00026]   In another example, calix[4]arene-nitrosonium complexes were obtained upon

25   mixing compound **1**, **2** or **5** with an excess $NO^+SbF_6^-$ in dry $CHCl_3$. Complexes **1**·$NO^+SbF_6^-$

and **2**·$NO^+SbF_6^-$ formed within about 20 hours. The UV-vis, FTIR and ^{1}H NMR spectra were

identical with the respective complexes **7** and **8**. Complex **5**·$NO^+SbF_6^-$ formed immediately

upon mixing. ^{1}H NMR results were: δ 6.54 (s, 8 H), 3.93 (t, J = 7.5 Hz, 8 H), 3.76 (t, J = 7.5

Hz, 8 H), 3.44 (s, 8 H), 1.8-1.75 (m, 8 H), 1.9-1.8 (m, 8 H), 1.5-1.2 (m, 48 H), 0.95 (t, $J = 7$

Hz, 12 H), 0.89 (t, $J = 7.5$ Hz, 12 H); UV-vis (CHCl$_3$): λ_{max} 600; FTIR (CDCl$_3$):

ν (NO$^+$)1876.

Example for Preparing 25-Hydroxy-26,27,28-trihexyloxy-*p-tert*-butylcalix[4]arene

5   (compound 10).

[00027]   A mixture of 25,26,27,28-tetrahydroxy-*p-tert*-butylcalix[4]arene (about 4.0g, 6.2

mmol), freshly distilled anhydrous DMF (about 80 mL), Ba(OH)$_2$•8H$_2$O (about 6.8g, 21.6

mmol), and BaO (about 6.36g, 41.5 mmol) were stirred at room temperature for 15 minutes.

n-Hexylbromide (about 21 mL, 184 mmol) was added, and the suspension was stirred at

10   room temperature for another about 12 hours. The mixture was diluted with water (about 100

mL), and the product was extracted with CH$_2$Cl$_2$ (3 x 100 mL). The organic layer was

washed with water (2 x 100 mL), dried over Na$_2$SO$_4$ and evaporated under reduced pressure.

The residue was recrystallized from MeOH to give pure compound 10 as a white solid. Yield

69%; Melting Point: 134-136 degrees Centigrade. ^{1}H NMR results were: δ 7.11 (s, 2 H),

15   7.03(s, 2 H), 6.51 (d, $J = 2.3$ Hz, 2 H), 6.49 (d, $J = 2.3$ Hz, 2 H), 5.72 (s, 1 H), 4.36 (d, $J = 13$

Hz, 2 H), 4.32 (d, $J = 13$ Hz, 2 H), 3.89 (t, $J = 8$ Hz, 2 H), 3.78 (t, $J = 8$ Hz, 4 H), 3.22 (d, $J = $

13 Hz, 2 H), 3.16 (d, $J = 13$ Hz, 2 H), 2.3-2.2 (m, 2 H), 2.0-1.8 (m, 4 H), 1.4-1.3 (m, 18 H),

1.32 (s, 9 H), 1.31 (s, 9 H), 0.92 (t, $J = 7$ Hz, 3H), 0.90 (m, 6 H), 0.81 (s, 18 H).

Example for Preparing 25-[(Ethoxycarbonyl)methoxy]-26,27,28-trihexyloxy-*p-tert*-

20   butylcalix[4]arene (11).

[00028]   A mixture of calix[4]arene compound 10 (about 5.0g, 5.6 mmol) and Na$_2$CO$_3$

(about 10.0g, 94 mmol) in CH$_3$CN (about 150 mL) was refluxed for about 15 minutes, after

which ethyl bromoacetate (about 10 mL, 90 mmol) was added, and the reflux continued for

12 hours. The inorganic salts were filtered, and the solvent was evaporated. The residue was

25   dissolved in CH$_2$Cl$_2$ (about 100mL) and washed with water (3 x 50 mL). The solvent was

evaporated, and the product was recrystallized from MeOH. Yield 89%; Melting Point: 121-

123 degrees Centigrade. ^{1}H NMR results were: δ 6.91(s, 2 H), 6.90 (s, 2 H), 6.63 (d, $J = 2$

Hz, 2 H), 6.61 (d, $J = 2$ Hz, 2 H), 4.86 (s, 2 H), 4.66 (d, $J = 12.5$ Hz, 2 H), 4.38 (d, $J = 12.5$

Hz, 2 H), 4.18 (q, J = 7 Hz, 2 H), 3.83 (t, J = 8 Hz, 4 H), 3.75 (t, J = 8 H, 2 H), 3.15 (d, J = 12.5 Hz, 2 H), 3.10 (d, J = 12.5 Hz, 2 H), 2.2-2.1 (m, 2 H), 2.0-1.9 (m, 4 H), 1.4-1.3 (m, 18 H), 1.27 (t, J = 7 Hz, 3 H), 1.18 (s, 9 H), 1.17 (s, 9 H), 0.96 (s, 18 H), 0.9-0.8 (m, 9 H).

Example for Preparing 25-(Carbomethoxy)-26,27,28-trihexyloxy-*p-tert*-butylcalix[4]arene (compound **12**).

[00029] A mixture of compound **11** (about 2.0 g 2 mmol), THF-H$_2$O, about 5:1 (100 mL) and KOH (about 1.0 g, 17.8 mmol) was refluxed for about 12 hours. The pH was adjusted to about 4 with aqueous 2 M HCl. The product was extracted with CH$_2$Cl$_2$ (2 x 50 mL), and the organic layer was dried over Na$_2$SO$_4$ and evaporated to give compound **12** as a white solid. Yield >95%; Melting Point: 136-137 degrees Centigrade. ^{1}H NMR results were: δ 11.28 (s, 1 H), 7.16 (s, 2 H), 7.14 (s, 2 H), 6.59 (d, J = 2.5 Hz, 2 H), 6.49 (d, J = 2.5 Hz, 2 H), 4.67 (s, 2 H), 4.45 (d, J = 12 Hz, 2 H), 4.23 (d, J = 12 Hz, 2 H), 4.08 (t, J = 7 Hz, 2 H), 3.8-3.7 (m, 4H), 3.24 (d, J = 12 Hz, 2 H), 3.16 (d, J = 12 Hz, 2 H), 1.95-1.8 (m, 6 H), 1.45-1.2 (m, 18 H), 0.90 (t, J = 7 Hz, 9 H), 0.83 (s, 18 H); MALDI-TOF MS, *m/z* 959.2 (M$^+$, calculated for C$_{64}$H$_{94}$O$_6$ 960.4). Anal. calculated for C$_{64}$H$_{94}$O$_6$: C 80.12; H 9.88. Found: C, 80.27; H, 9.88.

Example for Preparing *N*-Succinimide Ester of 25-(Carbomethoxy)-26,27,28-trihexyloxy-*p-tert*-butylcalix[4]arene (compound **13**).

[00030] A suspension of compound **12** (about 1.0g, 1.05 mmol), *N*-hydroxy succinimide (about 1.0g, 8.70 mmol), DCC (210 mg 1.05 mmol) and DMAP (about 40 mg, 0.32 mmol) in THF (about 50 mL) was stirred at room temperature for about 12 hours under nitrogen. After filtration, the solvent was evaporated, and the residue was redissolved in hexane (50 mL) and filtered again. The hexane solution was evaporated and the residue was purified by column chromatography (about CH$_2$Cl$_2$-hexane, 1:1) to afford compound **13** as a white solid. Yield 83%; Melting Point: 75-78 degrees Centigrade; ^{1}H NMR results were: δ 6.95 (s, 2 H), 6.92 (s, 2 H), 6.62 (d, J = 2.5 Hz, 2 H), 6.56 (d, J = 2.5 Hz, 2 H), 5.28 (s, 2 H), 4.56 (d, J = 13 Hz, 2 H), 4.41 (d, J = 13 Hz, 2 H), 3.89 (t, J = 8 Hz, 2 H), 3.79 (t, J = 8 Hz, 2 H), 3.75 (t, J = 8 Hz, 2 H), 3.18 (d, J = 13 Hz, 2 H), 3.12 (d, J = 13 Hz, 2 H), 2.82 (s, 4 H), 2.15-2.1 (m, 2 H), 1.95-1.85 (m, 4 H), 1.45-1.35 (m, 18 H), 1.20, 1.19 (2 x s, 18 H), 0.93 (s, 18 H), 0.9-0.8 (m, 9 H). Ester compound **13** appeared to be relatively unstable; attempts to obtain the MALDI-

TOF and/or CHN analytical data failed. The structure of compound **13** was confirmed through its transformation to calixarene amide compound **14**.

Example for Preparing 25-[(*n*-Octylcarbamoylmethoxy)-26,27,28-trihexyloxy-*p-tert*-butylcalix[4]arene (compound **14**).

5   [00031]   To the solution of ester compound **13** (about 75 mg, 70 μmol) in THF (about 10 mL), *n*-octylamine (about 18 mg, 0.14 mmol) and Et$_3$N (about 70 mg, 0.7mmol) were added, and the reaction mixture was stirred at room temperature for about 12 hours. The precipitate was filtered off, and the solution was evaporated. The residue was redissolved in CH$_2$Cl$_2$ (about 20 mL), washed with 2 M aqueous HCl (2 x 5 mL) and water (about 5 mL), and dried

10   over Na$_2$SO$_4$. The organic layer was then evaporated and the residue was recrystallized from CH$_3$CN to afford compound **14** as a white solid. Yield 65%. ^{1}H NMR results were: δ 8.43 (t, $J = 6$ Hz, 1 H), 6.99 (s, 2 H), 6.97 (s, 2 H), 6.57 (s, 4 H), 4.71 (s, 2 H), 4.38, 4.35 (2 x d, $J = 13$ Hz, 4 H), 3.9-3.7 (2 x m, 6 H), 3.42 (dt, $J = 6$ Hz, $J = 7$ Hz, 2 H), 3.23 (d, $J = 13$ Hz, 2 H), 3.14 (d, $J = 13$ Hz, 2 H), 2.0-1.8 (3 x m, 8 H), 1.5-1.3 (m, 30 H), 1.25 (s, 9 H), 1.23 (s, 9 H),

15   0.93 (s, 18 H), 0.95-0.85 (m, 12 H). FTIR (KBr): ν 3346 (NH), 2964, 1680 (C=O), 1537, 1473. Anal. calculated for C$_{72}$H$_{111}$NO$_5$•0.5CH$_3$CN: C, 80.35; H, 10.39; N, 1.93. Found: C 80.02; H 10.09; N, 1.91. Compound **14** was also independently synthesized from the acid chloride of compound **12** (prepared with SOCl$_2$), *n*-octylamine and Et$_3$N in CHCl$_3$.

Example for Preparing a Calix[4]arene Functionalized Silica Gel (**15**).

20   [00032]   A suspension of ester compound **13** (about 100 mg, 95 μmol), 3- aminopropyl-functionalized silica gel (Aldrich) (about 226 mg, 155 μmol) and Et$_3$N (about 92.9 mg, 0.92 mmol) in THF (50 mL) was stirred at room temperature for about 12 hours. The solid was filtered off, washed with CH$_2$Cl$_2$, MeOH, water, MeCN, and THF, and then dried under reduced pressure for about 3 days to provide a white powder. FTIR (KBr): ν 3383, 2960,

25   1650, 1556, 1477. Anal. Found for 3-aminopropyl silica gel (0.687 meq/g, C$_9$H$_{23}$NO$_3$Si): C, 7.42; H, 1.80; N, 1.91. Anal. Calcd for silica gel **15** (17% loading, 0.117 meq/g, C$_{73}$H$_{115}$NO$_8$Si): C, 15.43; H, 2.68; N, 1.82. Found: C 15.34; H 2.97; N, 1.70.

Example for Preparing *N*-Nitrosation of Amides by Calix[4]arene-Nitrosonium Complexes.
[It is noted that N-Nitrosoamides are potential carcinogens and should be treated with
extreme care.]

[00033] Complex **8** (about 1 equivalent) was added to the solution of amide

5   AlkC(O)NHMe **16a-c** (about 2-3 equivalents) in freshly distilled $CHCl_3$, and the reaction

mixture was stirred at room temperature for about 5 hours. The solvent was evaporated, and

the residue was analyzed by ^1H NMR spectroscopy and further separated by preparative

TLC. The spectral data for the obtained *N*-nitroso compounds AlkC(O)N(NO)Me **17a-c**

were identical with those independently obtained from AlkC(O)NHMe and NO_2/N_2O_4 or

10    $NO^+SbF_6^-$, and similar to results observed by others. (See, e.g., Garcia, J., et al. J. Org. Chem.

1984, *49*, pp. 3322-3327) For $CH_3(CH_2)_6C(O)NHCH_3$ (**16b**), ^1H NMR results were: δ 6.13

(bs, 1 H, NH), 2.73 (d, $J = 5$ Hz, 3 H, N-CH$_3$), 2.13 (t, $J = 7.5$ Hz, 2 H, C(O)CH$_2$), 1.6-1.5

(m, 2 H, CH$_2$), 1.3-1.1 (m, 8 H, CH$_2$), 0.83 (t, $J = 7.5$ Hz, 3 H, CH$_3$). For

$CH_3(CH_2)_6C(O)N(NO)CH_3$ (**17b**), ^1H NMR results were: δ 3.13 (t, $J = 8$ Hz, 2 H, C(O)CH$_2$),

15   3.03 (s, 3 H, N(NO)-CH$_3$), 1.8-1.7 (m, 2 H, CH$_2$), 1.4-1.2 (m, 8 H, CH$_2$), 0.84 (t, $J = 7.5$ Hz,

3 H, CH$_3$).

General Procedures for Synthesis of Compounds and Complexes of the Present Invention.

[00034] Calix[4]arenes compounds **1** and **2**, possessing a *cone* and a *1,3-alternate*

conformation, respectively, were synthesized through *O*-alkylation of the corresponding

20   parent calix[4]arenes with *n*-hexylbromide (see SCHEME 1). In the synthesis of compound

1, NaH was employed as a base in hot DMF. The preparation of compound **2** may include

the two-step alkylation of de-*t*-butylated calix[4]arene with *n*-hexyl bromide, using

successively K_2CO_3 and then Cs_2CO_3 in boiling MeCN.

SCHEME 1

1 R = (CH$_2$)$_5$CH$_3$

2 R = (CH$_2$)$_5$CH$_3$
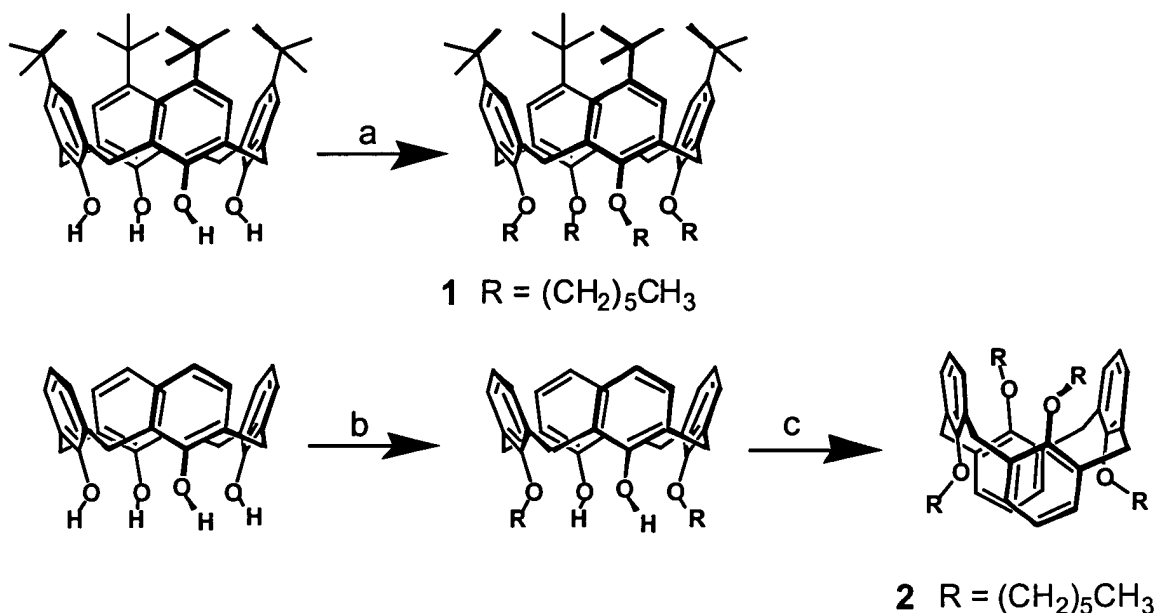
(a) n-hexyl bromide, NaH, DMF, 70 degrees Centigrade, 24 hours, 75% yield; (b) n-hexyl bromide, K$_2$CO$_3$, MeCN, 80 degrees Centigrade, 48 hours, 84% yield; (c) n-hexyl bromide, Cs$_2$CO$_3$, MeCN, 80 degrees Centigrade, 48 hours, 46% yield.

[00035] Bromination of compound **2** with NBS in acetone provides tetrabromocalix[4]arene compound **3** in a 53% yield (SCHEME 2). Tetrahydroxylated 1,3-alternate derivative compound **4** was obtained through bromo-lithium exchange in compound **3** (n-BuLi, THF, at –78 degrees Centigrade), followed by treatment with B(OMe)$_3$ and oxidation with H$_2$O$_2$ and aqueous NaOH (with a 40% yield after three steps). Calixarene compound **4** was subsequently alkylated with n-hexylbromide and NaH in hot DMF to yield octahexyloxycalix[4]arene compound **5** (about 85% yield). Mesitylene derived 1,3-alternate compound **6** was obtained for comparison, according to known procedures (see e.g., Pappalardo, S et al. *J. Org. Chem.* 1992, *57*, pp. 7102-7109 or Staffilani, M. et al. *Organometallics* 1998, *17*, pp. 1732-1740) and subsequent O-alkylation with *n*-hexylbromide (SCHEME 3).

SCHEME 2

2 → a → **3** → b,c,d → **4** → e → **5** R = (CH₂)₅CH₃
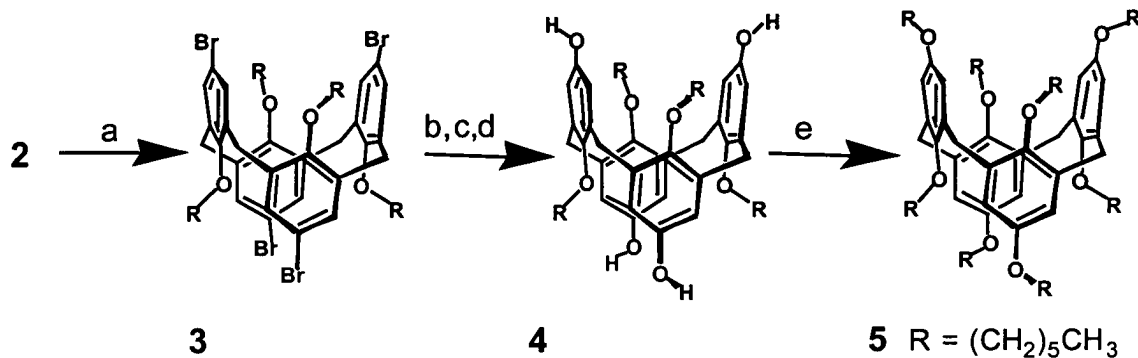
(a) NBS, acetone, room temperature, 48 hours, 53% yield; (b) *n*-BuLi (60 eq), THF, -78 degrees Centigrade, 0.5 hours; (c) B(OMe)₃, -78 to 0 degrees Centigrade; (d) H₂O₂, aqueous NaOH, -78 to 25 degrees Centigrade, 40% yield (three steps); (e) *n*-hexyl bromide, NaH, DMF, 70 degrees Centigrade, 24 hours, 85% yield.

SCHEME 3

6 R = (CH₂)₅CH₃
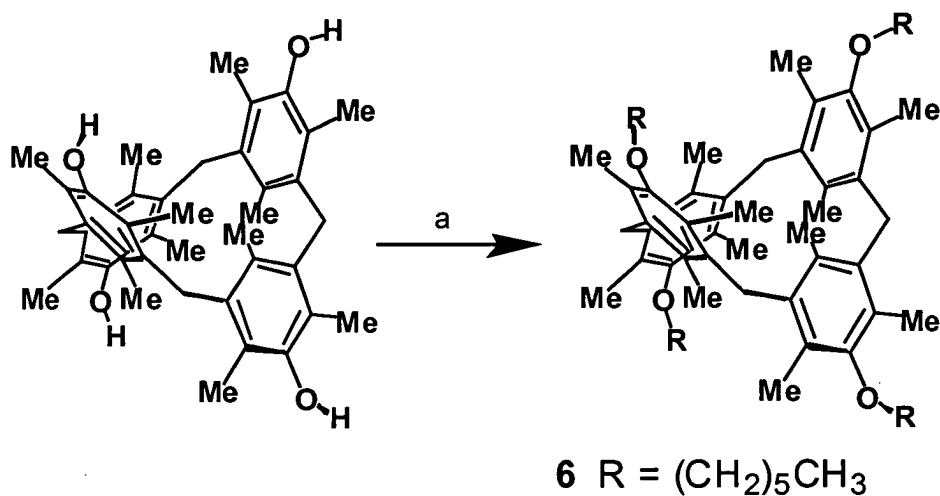
(a) *n*-hexyl bromide, NaH, DMF, 70 degrees Centigrade, 72 hours, 50% yield.

Interaction of Calix[4]arenes with Nitrogen Oxide Compounds of the Present Invention.

[00036] Bubbling NO₂ through the solutions of compounds **1, 2** and **5** in CHCl₃ resulted in instant identification of the complex, e.g., deep coloration. For example, solutions of compound **1** and compound **5** turned blue to dark blue, and a solution of compound **2** became purple to deep purple. The UV-vis spectra changed accordingly: the broad bands appeared at λmax of about 560, 512 and 600 nm for NO₂-exposed solutions of compounds **1, 2** and **5**,

16

respectively. This is in contrast to the colorless solution of compounds **1**, **2** and **5** and the yellow to pale yellow solution of NO_2 in $CHCl_3$ prior to mixing.

[00037] In the reactions with non-cyclic anisole (e.g., methoxybenzene), identification (e.g., coloration) occurred but was less pronounced upon exposure to NO_2. Moreover, when mesitylene-derived, Pappalardo's calixarene **6**, with the sterically *blocked* and conformationally much more *rigid* cavity, was tested, the complex was not detected (e.g., no coloration was observed). In compound **6**, the pair of methyl groups in the *ortho*-positions relative to the oxygen forces the methyl groups of the adjacent aromatic rings towards each other, not only blocking an access to the cylindrical inner cavity, but also significantly rigidifying it. The same effect takes place on the other side of the calixarene **6**, which makes its interior completely blocked. These reactions show the importance of *cavities* in the described transformations and illustrate NO^+ coordination inside **1**, **2** and **5**. (FIGURES 2, 3, and 4)

[00038] According to the molecular modeling performed on these compounds (e.g., using MacroModel 7.1), one NO^+ cation can fit inside the cavities of compounds **1**, **2** and **5**, and neither bent NO_2 nor bulky NO_3^- can be accommodated. Two parallel aromatic rings of a *cone* calix[4]arene participate in the NO^+ complexation, and all four rings of a *1,3-alternate* calixarene are involved. In mesitylene derived calixarene **6**, no NO^+ encapsulation may occur for steric reasons.

[00039] While an instant detection of a complex (e.g., nitrosated compounds **1**, **2**, and **5** via rapid coloration) shows charge-transfer between the prereactive nitrosating/nitrating species and the compounds (i.e., **1**, **2**, and **5**), the actual charge-transfer itself may be difficult to monitor. Interaction of NO_2 with compounds **1**, **2**, and **5** is very dynamic, and initial ^1H NMR analysis of the solutions showed complex, quickly changing pictures; the NO_2/N_2O_4 mixture is an effective nitrosating/nitrating agent. Not surprisingly, the NO_2-containing $CHCl_3$ solutions of compounds **1** and **2** decolored within or at about 1 to 2 hours, yielding mixtures of known *p*-nitrated calixarenes (preparative TLC, ^1H NMR). Calixarene **5**, much more activated for the electrophilic aromatic substitution, reacted with NO_2 more quickly—

as quickly as within a few minutes—producing a complex mixture of dealkylated and oxidized products.

[00040] When compounds 1 and 2 are simultaneously treated with $SnCl_4$ and 2 to 3 equivalents of NO_2, no nitration products are detected. Precipitation with hexanes resulted in detectable solids (e.g., deeply colored, moisture sensitive), assigned to nitrosonium complexes 7 and 8 (>90% yield, see FIGURES 2, 3, and 4). These complexes are very stable and can be stored, in the absence of moisture, for about several weeks, both in $CHCl_3$ solution and in the solid state. In one embodiment, complex 7 and 8 are identified by their color, wherein complex 7 is generally a dark blue and complex 8 is generally a deep purple.

Analysis and Characterization of Compounds and Complexes of the Present Invention.

[00041] Some spectral features of the obtained nitrosonium complexes are presented in the TABLE. The UV-vis spectra showed broad charge-transfer bands at λ_{max} of about 563 and 524 nm, and the FTIR spectra exhibited characteristic arene-NO^+ stretching at wavelengths of about $v = 1923$ cm^{-1} and 1955 cm^{-1} for complexes 7 and 8, respectively. The ^1H NMR spectra of complexes 7 and 8 exhibited new sets of the calixarene signals as well. (FIGURE 5) In particular, aromatic CH protons of guest-free compound 1 were seen as a singlet at 6.76 ppm. In nitrosonium complex 7, these were transformed into a singlet at 6.99 ppm. The methylene bridge CH_2 protons of compound 1 were recorded as doublets at 4.41 and 3.12 ppm ($J = 12.5$ Hz). In complex 7, these were seen as doublets at 4.39 and 3.44 ppm ($J = 13$ Hz). The aromatic protons of free compound 2 were seen as a doublet and a triplet, 2:1, at 6.92 and 6.68 ppm, respectively ($J = 7.5$ Hz). In nitrosonium complex 8, these were transformed into a triplet and a doublet, 1:2, at 7.17 and 7.08 ppm, respectively ($J = 7.5$ Hz). The methylene bridge CH_2 and OCH_2 protons of compound 2 were seen as a singlet and a triplet, 1:1, at 3.62 and 3.54 ($J = 7.5$ Hz), respectively. In complex 8, these were transformed into a singlet and a triplet, 1:1, at 3.60 and 3.87 ($J = 7.5$ Hz), respectively. Elemental analysis of the moisture sensitive and thermally unstable complexes 7 and 8 proved to be difficult but reproducibly showed the CHN ratios corresponding to the presence of only one NO^+ cation in both structures.

Table 1. Spectroscopic data for calix[4]arenes 1, 2, and 5, and their nitrosonium complexes.

Compound	δ, ppm	v, cm^{-1}	λ_{max}, nm
1	6.76 (s, arom), 4.41, 3.12 (2 x d, CH$_2$)		
2	6.92 (d, arom), 6.68 (t, arom), 3.62 (s, CH$_2$), 3.54 (t, OCH$_2$)		
5	6.55 (s, arom), 3.83 (t, OCH$_2$), 3.62 (s, CH$_2$), 3.35 (t, OCH$_2$)		
5•NO$^+$SbF$_6^-$	6.54 (s, arom), 3.93 (t, OCH$_2$), 3.76 (t, OCH$_2$),	1876	600
7	6.99 (s, arom), 4.39, 3.44 (2 x d, CH$_2$)	1923	563
8	7.17 (t, arom), 7.08 (d, arom), 3.87 (t, OCH$_2$)	1955	524

At 295 ± 1 K; The above reported spectral features of complexes 1•NO$^+$SbF$_6^-$, 2•NO$^+$SbF$_6^-$, and 5•NO$^+$SbF$_6^-$ are similar to those of complexes 7, 8, and 9, respectively; In CDCl$_3$ for ^{1}H NMR and FTIR, and in CHCl$_3$ for UVvis.

5   [00042]   Independent structural evidence came from the complexation reactions between calixarenes 1 and 2 and commercially available NO$^+$SbF$_6^-$ salt. (FIGURE 2 and 3) Specifically, the CDCl$_3$ solutions of compounds 1 and 2 were treated with NO$^+$SbF$_6^-$ at about 295 K and the complexation induced changes in the UV-vis, FTIR and ^{1}H NMR spectra (as recorded). Under these conditions, the complexation process proved to be rather slow,

10   however after about 20 hours no starting calixarenes 1,2 were observed and the corresponding UV-vis, FTIR and ^{1}H NMR spectra exhibited features similar to those of nitrosonium complexes 7,8.

[00043]   Analogously, a nitrosonium complex of calixarene 5 was obtained. The UV-vis spectrum of the detected complex 5•NO$^+$SbF$_6^-$ (e.g., visible as dark blue) showed broad band

15   at about $\lambda_{max} \sim 600$ nm, and the FTIR spectrum exhibited characteristic arene-NO^+ stretching at about 1876 cm^{-1}. The ^{1}H NMR spectrum of 5•NO$^+$SbF$_6^-$ is different from complex 5 as well. For example, the methylene bridge CH$_2$ and both OCH$_2$ protons of complex 5 were seen as a singlet and two triplets, 1:1:1, at 3.62, 3.83 ($J = 7$ Hz), and 3.35 ppm ($J = 7$ Hz),

respectively. In complex $5\cdot NO^+SbF_6^-$, these were observed at 3.44, 3.93 ($J = 7.5$ Hz), and 3.76 ppm ($J = 7.5$ Hz), respectively.

[00044] The association constants for the above complexes were too high to be measured by the ^{1}H NMR technique. Even slight excess of $NO^+SbF_6^-$ results in the complete complex formation in CDCl$_3$, and no free calixarenes **1, 2,** and **5** were observed after equilibration. The K_{ass} values $>10^6$ M^{-1} ($\Delta G^{295} > 8$ kcal mol^{-1}) for the complexes were estimated and in agreement with published values in CH$_2$Cl$_2$ (e.g., see Rathore, R. et al. *Angew. Chem. Int. Ed.* 2000, vol. 39, pp. 2123-2127). While complexes $1\cdot NO^+SbF_6^-$ and $2\cdot NO^+SbF_6^-$ formed slowly, over about 20 hrs, it took only a few minutes to form complex $5\cdot NO^+SbF_6^-$. Moreover, highly electrophilic NO^+ tended to further react with $5\cdot NO^+SbF_6^-$, and unidentified impurities were seen in the NMR spectrum after several minutes. Unlike complexes $1\cdot NO^+SbF_6^-$ and $2\cdot NO^+SbF_6^-$, which are chemically stable for weeks, complex $5\cdot NO^+SbF_6^-$ decomposed within a day (as observed by ^{1}H NMR).

[00045] Addition of H$_2$O or MeOH to the freshly prepared CHCl$_3$ solutions of complexes **7** and **8** and the nitrosonium complexes prepared from **1, 2** and **5** and $NO^+SbF_6^-$, resulted in the complete complex dissociation and recovery of calixarenes **1, 2** and **5** (as observed by preparative TLC, UVvis, ^{1}H NMR).

[00046] Spectral data for the reactions between compounds **1** and **2** and NO$_2$, in the absence of SnCl$_4$ were re-observed. As excess NO$_2$ was passed through the solution of compound **1**, nitrosonium complex $[1\cdot NO^+]NO_3^-$ was clearly seen (UVvis, ^{1}H NMR), along with the mixture of nitration products. For compound **2**, no signals for nitrosonium complex $[2\cdot NO^+]NO_3^-$ were detected, because, while formed, these nitrosonium species quickly react with an excess NO$_2$. Both reactions subsequently yield *p*-nitrated calixarenes.

[00047] As such, reactions between NO$_2$/N$_2$O$_4$ and *O*-alkylated calix[4]arenes proceed via the NO^+ encapsulation. In addition, calix[4]arene-nitrosonium complexes can be significantly stabilized by Lewis acids.

[00048]   Host-Guest Dynamics of the Present Invention.

[00049]   As evident from the ^{1}H NMR data, the NO^+ exchange in and out of the cavity is slow on the NMR time scale. For example, in the titration experiments between calixarenes 1 and 2 and $NO^+SbF_6^-$, both free and complexed species can be observed separately. This is typical for the host-guest complexes with high $K_{ass} > 10^6$ M^{-1} values. On the other hand, the NO^+ guest, with the van der Waals dimensions <2 Å, freely migrates within the cavity at room temperatures (FIGURE 6). Indeed, the ^{1}H NMR spectra of complexes 7,8 possess the same symmetry as guest-free calixarenes 1,2, which in principle should be reduced upon complexation with nonsymmetrical NO^+.

[00050]   *Cone* calix[4]arene complex 7 should have a pinched, C_{2v} symmetrical conformation, since only two opposite, cofacial aromatic rings trap NO^+. However, the observed at room temperature NMR spectrum exhibits a C_{4v} symmetry, indicating a fast (on the NMR time scale) exchange between two C_{2v} structures.

[00051]   *1,3-Alternate* calix[4]arene complex 8 should exhibit a C_{2v} symmetry, with two different top and bottom halves of the skeleton. Instead, the observed symmetry is S_4, with equal top and bottom halves.

[00052]   In one aspect of the present invention, the complexation process is reversible, and the NO^+ guest can readily leave the calixarene cavity. For example, addition of H_2O to the freshly prepared $CHCl_3$ solutions of complexes 7,8 resulted in the complete dissociation and recovery of calixarenes 1,2. Complex 8 generally decolored within seconds; complex 7 may take several minutes to decompose. Kinetics factors appears to be responsible for these observations, where *t*-Bu groups at the upper rim of the latter complex pose significant steric hindrances and protect the encapsulated NO^+ species from the entering H_2O. Such stability of the arene-NO^+ complex is without precedent.

[00053]   In another aspect of the present invention, NO^+ guest can be transferred from one calixarene container to another (FIGURE 7). Calixarene 5 was specifically designed to

promote such transfer from the preformed complex **8**. Four additional, electron donating O(CH₂)₅CH₃ groups were introduced in *p*-positions to the initial set of $O(CH_2)_5CH_3$ groups. This makes cavity of compound **5** significantly more π-electron rich and dramatically increases its affinity towards positively charged NO^+.

5   **[00054]** Examples of the exchange include obtaining complex **8** by treating calixarene **2** with 3 equivalents NO_2 and 1 equivalent $SnCl_4$ in $CHCl_3$. Further, complex **8** and "empty" host **5** were mixed in an 1:1 ratio at 295 K in dry chloroform, and the UV-vis and ^1H NMR spectra were recorded over two hours. Due to the strong affinity of **2** towards NO^+, the guest presence outside the cavity, in a bulk solution, was considered negligible; the only source of

10   NO^+ was complex **8**. Initially, the ^1H NMR spectrum exhibited only sets of signals for complex **8** and free calixarene **5**, and the corresponding UV-vis spectrum showed only the characteristic absorption for charge-transfer in complex **8**. Within minutes, however, the guest transfer was clearly detected. The band at λ_{max} of about 524 nm, assigned to complex **8**, systematically decreased and a new band at λ_{max} of about 600 nm, corresponding to new

15   complex **9**, appeared.

[00055] The nitrosonium transfer can be identified visually. For example, a purple solution of complex **8** in $CHCl_3$ turns blue upon addition of calixarene **5**. (FIGURE 8) When followed by ^1H NMR spectroscopy, the NO^+ exchange resulted in clean transformation of the spectra from mixture **8** + **5** to mixture **2** + **9**. (FIGURE 9) Note that the residual $CHCl_3$

20   signals in FIGURE 9 are marked with a dark circle and the decomposition products of compound **5** are marked with an asterisk The methylene bridge CH₂ and OCH₂ protons of complex **8**, seen as a singlet and a triplet, 1:1, at 3.60 and 3.87 ppm ($J = 7.5$ Hz), may slowly decrease in intensity. Instead, two OCH₂ triplets at 3.93 and 3.76 ppm ($J = 7.5$ Hz) and the methylene bridge CH₂ singlet at 3.44 ppm appear and grow (assigned to complex **9**).

25   Complex **9** was confirmed in a series of independent experiments between **5** and $NO^+SbF_6^-$. Signals for "empty" calixarene **2** also appear, although slightly shifted due to the presence of $SnCl_4$, and signals for complex **8** disappear. Within about an hour the nitrosonium transfer may be completed; both the ^1H NMR and UV-vis spectra exhibited only the signals of complex **9** and free calixarene **2**. No traces of initial complex **8** were detected.

[00056]   This is a new example of a quantitative guest transfer between two different molecular containers. As such, molecular containers of the present invention serve as storage for information and processing, especially because they can be readily detected (e.g., by color changes) when the compounds are switched between two distinguishable states. In addition to switching from a free and entrapped state with the calixarene complexes of the present invention, the nitrosonium may be further transferred to another substrate (e.g., solid support, solid interface, etc. that accepts nitrosonium).

Functionalized Silica Gel of the Present Invention.

[00057]   In the present invention, the interaction of calix[4]arene and a nitrosonium ion is: (a) reversible, (b) detectable (e.g., by color changes) and (c) effective for purifying NO_x compounds in the presence of other gases, such as H_2O, O_2, HCl, HBr, SO_X, and NH_3. In particular, these other vapors/gases are not involved in the same interactions/reactions with calixarenes. For example, neutral NO gas does not interact with calixarenes.

[00058]   Current NO_2 sensors are generally electrochemical and monitor changes in potential upon exposure of metal surfaces to NO_2. In many cases however, other vapors - H_2O, O_2, HCl, HBr, SO_X, and NH_3 significantly influence detector selectivity and therefore its sensitivity. Optical sensors, generally based on color changes in a reaction between NO_2 and certain organic compounds, are more selective as the reactions are specific. However, reversibility of these sensing devices is not easy to achieve.

[00059]   For application in any sensing technology, receptor molecules must not only be synthetically available, but also readily immobilizable on solid supports or surfaces. A wide variety of polymers and nanomaterials are commercially available. In one embodiment of the present invention, a solid support is functionalized with calixarene modules of the present invention (e.g., 3-aminopropylated silica gel). Molecular modeling of calix[4]arene fragments (of about 10 x 10 Å in dimension) attached to a solid support via the *lower* rim indicates the proper configuration of the *upper* rim to sufficiently respond to the presence of and to detect a gas analyte.

[00060]   Calixarenes may be successfully attached to a surface, wherein tris-*O*-substituted

calixarene **10**, prepared by selective alkylation of the parent calix[4]arene with *n*-

hexylbromide and $BaO/Ba(OH)_2$[29] in DMF (69%), was further alkylated with ethyl

bromoacetate to afford derivative compound **11** (Na_2CO_3, MeCN, 89%). (SCHEME 4)

5   SCHEME 4

10 R = $(CH_2)_5CH_3$

11 R' = OC_2H_5

12 R' = OH

13 R' = —O-N

14 R' = $NH(CH_2)_7CH_3$
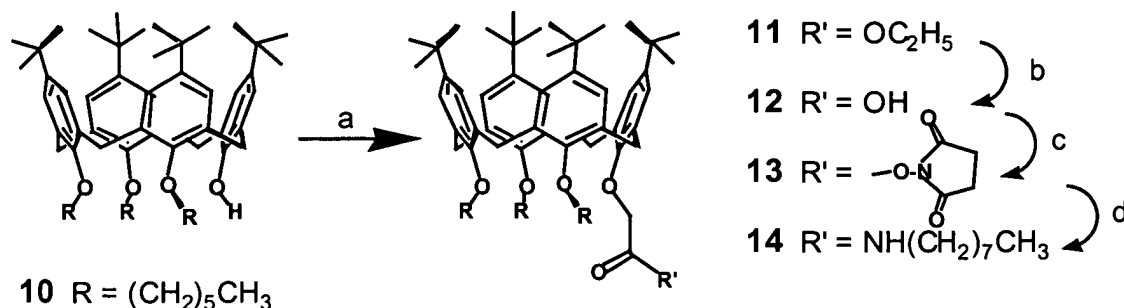
a) $BrCH_2C(O)OC_2H_5$, Na_2CO_3, MeCN, 80 degrees Centigrade, 12 h, yield of about 89 %; (b) KOH,

THF-H_2O, 100 degrees Centigrade, 12 h, then aqueous HCl, yield of about or >95%.; (c) *N*-hydroxy

succinimide, DCC, DMAP, THF, room temperature, 12 hours, yield of about 83%; (d) $CH_3(CH_2)_7$-

NH_2, Et_3N, THF, room temperature, 12 hours, yield of about 65%.

10   [00061]   As in SCHEME 4, derivative compound **11** was hydrolyzed with KOH in a THF-

H_2O mixture, resulting in calixarene acid **12** in a quantitative yield. Acid compound **12** was

further activated with *N*-bromosuccinimide (DCC, DMAP, THF) to afford active ester

compound **13** (yield of about 83%). Compound **13** readily reacts with amines. Thus, amide

14 was prepared from compound **13** and *n*-octylamine (Et_3N, THF, yield of about 65%) and

15   used for control experiments. Analogously, ester compound **13** was coupled to 3-

aminopropyl-functionalized silica gel in THF in the presence of Et_3N to afford material **15**.

(FIGURE 10) The presence of a calix[4]arene fragment in compound **15** was confirmed by

the FTIR analysis in KBr disks: v(CH) of about 2960 cm^{-1} and v(C=O) of about 1650 cm^{-1}

were recorded (similar to the stretching of model calixarene amide **14**: v(CH) = 2964 cm^{-1}

20   and v(C=O) = 1680 cm^{-1}). From the CHN analysis, about 17% calixarene loading was

achieved (there is steric bulkiness of the calixarene fragment).

[00062]   In the NO_2 entrapment demonstration, a stream of the gas was passed through Pasteur pipettes, loaded with silica gel **15**. (FIGURE 10) In one pipette, dry silica gel **15** was loaded, and the other contained **15** preliminary wetted with $CHCl_3$. With both silica gels, entrapment of a nitric oxide compound was instantly detected (e.g., color change, such as the gel turning dark purple) and indicated the NO^+ complexation. When the material was wetted, the identification may be quicker and more complexation may occur (e.g., the color of the wetted material appeared to be deeper and lasted for hours). The dry material decolored within minutes. The FTIR spectrum, recorded in KBr disks, gave weak but reproducible stretch at v of about $1920 \ cm^{-1}$, indicating the presence arene-NO^+ complexes. No detection was apparent for the pipette loaded with the starting solid support, e.g., 3-aminopropylated silica gel. For FIGURE 10, columns were prepared by (A) loading with starting aminopropyl functionalized silica gel, (B) loading with dry silica gel **15**, (C) loading with **15** and flashed with $CHCl_3$ and then flashing all three columns with NO_2 (about 30 seconds) followed by photographing columns about 2-3 minutes later. For the present invention, calixarene cavities play a role in the described processes. Indeed, a solid support such as silica gel **15** is applicable for NO_2 detection and may even be used for purification of other nitrogen oxide compounds, especially NO.

Nitrosating Reagents of the Present Invention.

[00063]   In addition to the previously described features of the present invention is the ability of calixarene-nitric oxide containing molecules. complex to act as nitrosating agents.

[00064]   It is known, that reactions between NO^+ generating agents (e.g., NOCl, N_2O_3, NO_2/N_2O_4, nitrosonium salts, etc.) and amides and short peptides proceed via N-nitrosation and yield biologically important nitroso-derivatives. Nitrosation of peptides may be used in analytical protocols, such as in protein sequencing, and occurs in biologic processes, when NO_X (e.g., as atmospheric pollutants) interacts with biological tissues and fluids. Here, the corresponding reaction mechanisms typically incorporate an electrophilic attack of NO^+ on a nucleophilic oxygen or nitrogen of the substrate.

[00065]   In yet another embodiment of the present invention, a sample such as *1,3-alternate* based nitrosonium complex **8** was mixed with AlkC(O)NHMe **16a-c** (Alk = *n*-Pr, *n*-Hex, *n*-Hept) in dry CHCl$_3$ and stirred at room temperature for several hours. (FIGURE 11) The corresponding *N*-nitrosoamides AlkC(O)N(NO)Me (**17a-c**) were formed in about 30-40%

5   yield. Under the same conditions, the *cone* calixarene complex **7** reacted very slow, and only traces of the *N*-nitrosoamide products were detected by ^{1}H NMR spectroscopy. Likewise with water, *t*-Bu groups at the upper rim of complex **7** impose steric hindrances and protect the encapsulated NO$^+$ species from the substrate. The rate limiting formation of the nitroso intermediates should take place within the calixarene cavity, prior to the NO$^+$ dissociation.

10   Once formed, these sterically bulky species leave the interior, and undergo further transformations in bulk solution.

[00066]   These transformations provide additional structural evidence for the calixarene-NO$^+$ complexes of the present invention and illustrate the utility of calixarenes as supramolecular/encapsulated nitrosating reagents (e.g., complexes as encapsulated reagents

15   for nitrosation). As used herein, encapsulated reagents of the present invention are highly reactive species, reversibly entrapped within a host' cavity that may be released to the reaction mixture via one or more methods. The cavity offers protection from the bulk environment and thus controls the reaction rates. Reactions with encapsulated reagents may occur either within the cavity interior, or outside, upon release. Some of the more critical

20   factors responsible for reagent release and for the reaction to occur are noncovalent forces holding the molecule-within-molecule complex together, such as temperature, solvent polarity and the substrate-cavity size-shape fit. Indeed, with deeper calixarenes, where the cavities could accommodate the reaction intermediates, supramolecular effects in these processes are more pronounced.

25   [00067]   The present invention provides a new and improved NO$_x$ sensing and fixation process that employs simple calix[4]arenes. With the present invention, calixarenes provide and transmit information about NO$_x$ binding that can be readily detected (e.g., via visible light signals, electrochemical changes, spectroscopic changes), wherein the charge-transfer interactions allow the detection of various forms of nitrogen oxide compounds in the

presence of other gas mixtures, such as H_2O, O_2, HCl, SO_X, NH_3, NO, as well as chemical mixtures produced by chemical processing and manufacturing. The calixarenes and complexes of the present invention may be immobilized, in solution or attached to a ligand. The resulting calixarene-nitrogen oxide compound complexes may be used as vehicles for

5   nitrosonium transfer and encapsulated nitrosating reagents. NO_2/NO_X sensing molecules and as storing materials.

[00068]   Additional objects, advantages and novel features of the invention as set forth in the description, will be apparent to one skilled in the art after reading the foregoing detailed description or may be learned by practice of the invention. The objects and advantages of the

10   invention may be realized and attained by means of the instruments and combinations particularly pointed out here.